

REMARKS

Claims 1-85 were present in the application as filed, with claims 69-85 cancelled by preliminary amendment. In an amendment filed in response to a restriction dated March 17, 2008, claims 1-68 were cancelled and new claims 86-107 were presented. In the present amendment, claims 86-107 are amended and new claim 108 is presented. Claims 86-108 are pending in the application.

Claim 86 is amended to specify that the subject method for monitoring protein synthesis comprises:

providing a system comprising a marker detectable through detection of electromagnetic radiation, the marker comprising a first label covalently bound to at least one ribosome or a fragment thereof, and a second label covalently bound to at least one element selected from the group consisting of a different location of the ribosome or fragment thereof and a tRNA;

detecting electromagnetic radiation signals emitted from the system in response to protein synthesis activity; and

analyzing said emitted radiation so as to identify at least one protein being synthesized.

The final step recited in amended claim 86 incorporates a limitation previously recited in claim 101; claim 101 is canceled. Claims 90 and 94 as amended recite language that is consistent with claim 86 as amended. Claim 94 is further amended to recite “one” in the phrase “at least one photo-active component”, so as to be consistent with base claim 89. Claim 91 is amended by deletion of subject matter, so as to be consistent with the deletion of “an amino acid” in claim 86. Claims 92 and 102 are amended to correct the dependencies. New claim 108 depends from claim 99 and specifies that the single ribosome detected is in a live cell. Claim 88 is canceled.

Support for the amendments to claim 86 is found in the specification, for example at paragraphs [0027], [0029], [0044], [0049], [0078], [0184]-[0192], [249]-[251] of the published application. Support for claim 108 is found in the specification, for example at

paragraphs [0179] and [0280].

Priority

The relevance of the second and third paragraphs of item 2 on page 2 of the Office Action is not clear to Applicants. The present application is a 371 filing of PCT International application no. PCT/IL2003/001011 filed November 27, 2003, which claims the priority of United States application no. 60/429,532 filed November 29, 2002. The priority PCT application was amended to include this information as a “Cross-Reference to Related Applications” by preliminary amendment filed with the national stage application. The Examiner is kindly requested to acknowledge that the priority for the present application has been properly identified in the present application.

Specification

The specification was objected to as containing embedded hyperlinks or other browser-executable code. The specification is amended above to convert the embedded hyperlinks to url addresses, which are not browser executable.

Rejections under 35 U.S.C.102(b)

Claims 86-95 are rejected under 35 U.S.C.102(b) as anticipated by Rothschild et al. (US patent 6,210,941; hereinafter referred to as “Rothschild”) or Dirks et al. (Histochemistry and Cell Biology Vol. 115, No.1, January 2001, p. 3-11; hereinafter referred to as “Dirks”).

Rothschild discloses and teaches methods for the labeling, detection, quantitation and isolation of nascent proteins produced in a cell-free or cellular translation system without the use of radioactive amino acids. The invention of Rothschild is based on the use of misaminoacylated tRNAs, which Rothschild characterizes as tRNA molecules charged with anything but a native amino acid molecule, such as a native amino acid

coupled with a chemical moiety, a non-native amino acid, an amino acid derivative or analog, or other detectable chemicals, any of which are referred to as "markers". The misaminoacylated tRNAs incorporate their markers into the growing peptide chain during translation forming labeled nascent proteins which can be detected and isolated by the presence or absence of the marker (see col. 6, line 63-col. 7, line 46).

Rothschild further teaches that the invention may utilize more than one type of marker, specifically amino acids bearing distinct reporter molecules in which the interaction between the reporters causes them to be specifically detectable, for example by resonant energy transfer. Rothschild discloses that such interactions require close proximity of the reporters, which may be enhanced by choosing tRNA species that will insert markers into positions that are close to each other in the structure of the protein. According to Rothschild, such reporters enable the detection of proteins without purification or isolation from the synthesis system, and are further useful for determining the rate of synthesis of proteins and for optimizing synthesis (col. 18, lines 22-65).

The presently claimed method is distinguishable over Rothschild in a number of ways. In particular, claim 86 as amended requires providing a marker which comprises a first label covalently bound to at least one ribosome or a fragment thereof.

Rothschild provides no teaching or suggestion of a label covalently bound to a ribosome or fragment thereof. The Office Action asserts that a labeled tRNA as disclosed in Rothschild reads on a labeled ribosome fragment. However, a misaminoacylated tRNA as taught by Rothschild does not and cannot result in a label being or becoming covalently bound to a ribosome or fragment thereof, upon addition to a protein translation system. One of average skill in the art is aware that in the process of protein translation, tRNA molecules associate with ribosomes, specifically in the A, P and E sites. This association however, is not through covalent bond formation, but rather through Watson-Crick base pairing between the anti-codon of the tRNA and codons of the mRNA, as described for example in "Molecular Biology of the Cell", fourth edition, Garland Science, page 344, attached hereto as Appendix A. Alternately or in addition, a large

body of evidence exists that the tRNA codons form base pairs with RNA nucleotides in a ribosomal cavity termed the peptidyltransferase center (PTC) containing the A and P sites, as disclosed for example in Agmon et al, Eur. J. Biochem. 270, 2543-2556 (2003), attached hereto as Appendix B (see in particular pages 2543, col. 2; pages 2545-2547).

Since a misaminoacylated tRNA as provided by Rothschild will not become covalently bound to ribosome via its interaction therewith in a protein synthesis system, the disclosure of Rothschild does not anticipate a label covalently bound to a ribosome or fragment thereof, as currently claimed. Furthermore, according to the subject application, a label covalently bound to ribosomes may be obtained by various strategies, for example by coupling ribosomal proteins with organic dyes or by creation of fusion proteins with a fluorescent protein (see [0205]-[0208] of the published application). No such methods are taught or suggested by Rothschild.

The method of the present invention is further distinguished over Rothschild in that the subject marker does not and cannot become incorporated into the nascent peptide chain. This is opposite from the situation of Rothschild, in which each of the embodiments includes the express requirement that the marker incorporates into the nascent peptide chain (see for example col. 4, line 51-col. 5, line 40). More specifically the presently claimed marker comprises a first label covalently bound to a ribosome or fragment thereof, and a second label covalently bound to at least one of: a different location of the ribosome or fragment thereof and a tRNA. None of the possible combinations i.e. labeled ribosome plus labeled-ribosome or labeled-ribosome plus labeled-tRNA can result in label becoming incorporated into the nascent protein when present together in a protein translation system.

The present invention is further distinguished over Rothschild in that the final step recited in claim 86 as amended is directed to analyzing the emitted radiation so as to identify at least one protein being synthesized. This step, as disclosed in the subject application for example at paragraphs [0249]-[0251] and [0305]-[0314] of the published application, may involve correlating emitted signals with a protein database so as to

obtain protein identification data.

In contrast, Rothschild provides no teaching whatsoever of a step or means of identifying a protein. Rothschild teaches labeling, detecting, quantitating and isolating nascent proteins but does not and cannot provide a means of establishing the identity of a produced protein using analysis of radiation emitted from the protein translation system. In fact, Rothschild teaches that identity of a target protein should be established before carrying out labeling of amino acids. For example, for incorporating reporters in close proximity in a protein sequence, *a priori* knowledge of the amino acid sequence is required so that suitable aminoacetylated tRNA pairs e.g. tyrosine-tRNA and tryptophan-tRNA may be used (see col. 18, lines 43-51). Rothschild further refers to optimizing production in a protein translation system by monitoring the rate of production of a specific calibration protein, and studying gene regulation, activities which require prior knowledge of the identity of the protein being labeled/monitored, and do not provide a means of identifying a protein being synthesized on the basis of analysis of emitted radiation.

For at least the aforementioned reasons, the presently claimed invention is distinguishable over Rothschild and not anticipated.

Dirks generally discloses methods of labeling RNA molecules so as to study RNA synthesis and subsequent transport and processing. Applicants respectfully traverse the assertion in the Office Action that Dirks relates to methods for visualizing RNA processing in ribosomes which is viewed as monitoring of protein synthesis. Dirks in fact, is directed primarily to the earlier events of RNA transcription and RNA localization in the nucleus, as further detailed below. Dirks however, does provide one specific example of RNA detection in cytoplasm, namely that using a FRET approach with fluorescently labeled oligodeoxynucleotides for detection of c-fos mRNA in transfected cells, as demonstrated by Tsuji et al Biophysical Journal Vol. 78 June 2000, 3260-3275 (hereinafter referred to as "Tsuji", a copy of which is attached).

The presently claimed method differs from Dirks in several ways. First, Dirks provides no disclosure of a marker comprising a first label covalently bound to a ribosome or fragment thereof, and a second label covalently bound to one of: a different location of the ribosome or fragment thereof, or a tRNA, as required by the present invention. Rather, the FRET method taught by Dirks utilizes two different oligonucleotide probes which hybridize on the same mRNA target molecule (see legend to Fig. 3F). More specifically, complementary base-pairing which is the basis of nucleotide hybridization for attachment of the label of Dirks, is distinct and different from covalent attachment of a label as in the present invention. In addition, the probe of Dirks labels only mRNA, which corresponds to none of the ribosome, ribosome fragment or tRNA specified in claim 86.

Furthermore, Dirks provides no indication that in the disclosed FRET approach, electromagnetic signals are emitted from the system in response to protein synthesis activity. Rather, Dirks discloses that the fluorescence is generated by two hybridization events on the mRNA.

In addition, there is no teaching or suggestion in Dirks that the mRNA detected is involved in protein synthesis or that label is in any way associated with ribosomes. Dirks merely teaches that the probes used were oligodeoxynucleotides bound to streptavidin "to prevent their passage through nuclear pores" (page 9, col. 2). Importantly, review of Tsuji indicates that the original article which Dirks cites provides no teaching or suggestion that the labeled probes are in any way associated with ribosomes. In fact, Tsuji discloses that the streptavidin-bound oligoDNA probes hybridized with the target immediately after injection to the cytoplasm, and that the results suggest that "most of the streptavidin-bound oligoDNAs did not have any specific interaction with other components in cells and were free within the cytoplasm" (see Tsuji page 3273, col. 1).

Finally, Dirks fails to disclose analysis of emitted signals so as to identify at least one protein being synthesized, since Dirks relates only to detecting a target mRNA corresponding to a protein of known identity. Dirks indicates that two hybridization

events have to occur in order to generate a fluorescent signal but provides no indication that the signal can be used to identify a protein. Dirks merely states that the detection of FRET may be improved by FLIM.

For at least the aforementioned reasons, the presently claimed invention is distinguishable over Dirks and not anticipated.

Based on the above, neither Rothschild nor Dirks disclose each and every element of claim 86 as amended, or the additional rejected claims. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C.102(b) be withdrawn.

Rejections under 35 U.S.C.103(a)

Claims 86 and 96-107 are rejected under 35 U.S.C.103(a) as unpatentable over Rothschild or Dirks.

Rothschild contains the explicit teaching that the marker is incorporated into the nascent protein (see Summary of the Invention), a feature which as indicated above, does not even occur using the claimed invention. In fact, incorporation of marker into the nascent protein as taught by Rothschild would be detrimental to the working of the current invention, a major advantage of which is the capability to identify the proteins synthesized in a protein synthesis system, in particular a cell under various conditions, as disclosed for example at paragraphs [0030]-[0033].

Further, Rothschild's teaching of two different reporter markers does not suggest or render obvious the subject invention, since Rothschild teaches that the proximity of such markers on the same peptide chain is important for their interaction and specific detection: "Although these markers are also present on the misaminoacylated tRNAs used for their incorporation into nascent proteins, the interaction of the markers occurs primarily when they are incorporated into protein due to their close proximity" (see col. 18, lines 22-51). Thus, according to Rothschild, detecting of emitted electromagnetic radiation is dependent on the presence of reporter markers on closely spaced amino acid

residues within a protein, and detectable signals are not obtained merely by providing other labeled macromolecules required for protein synthesis, such as for example, tRNA.

The present method uses a marker comprising a first label covalently bound to the ribosome, and a second label covalently bound to one of: a different location of the ribosome or fragment thereof, or a tRNA. Significantly, none of the labeled combinations encompassed by the presently claimed invention results in the incorporation of two different labels (i.e. reporters) on proximal amino acid residues within a nascent protein. In fact, in the present invention, the disclosed and claimed embodiments of R-T labeling (see paragraphs [0183]-[0186]) and R-R labeling (see paragraphs [0190]-[0196]) cannot result in incorporation of even a single label into an amino acid residue in the nascent protein.

Accordingly, on the basis of Rothschild one of average skill in the art would not be motivated to provide a marker as currently claimed, since the current method cannot yield a single label on a peptide chain, and certainly not two labels in close proximity on a peptide chain, as required by Rothschild.

Furthermore, the teachings in Rothschild regarding insertion of markers into positions that are close to each other in the primary, secondary or tertiary structure of the protein (col. 18, lines 43-51), and regarding optimization of protein synthesis by monitoring the rate of production of a specific calibration protein are steps which require prior knowledge of the sequence and/or structure of the protein being detected. In contrast, the present method does not require any such information and in fact provides a means of identifying any protein being synthesized without *a priori* preparations. Thus, Rothschild cannot and does not suggest to one of average skill in the art a step of analyzing electromagnetic radiation emitted from the system so as to identify the protein being synthesized.

For at least the foregoing reasons, Rothschild does not render obvious the presently claimed invention.

As indicated above, Dirks does not provide any teaching or suggestion of a covalently labeled ribosome or fragment thereof, or of analyzing emitted signals so as to identify a protein being synthesized. In fact, Dirks provides no teaching whatsoever concerning protein synthesis, but is directed primarily to the earlier events of RNA transcription and RNA localization in the nucleus.

More specifically, Dirks discusses experiments involving Br-UTP labeling of nascent transcripts which “revealed that RNAs are synthesized throughout the nucleoplasm...with the exception of ribosomal RNA (rRNA) which is synthesized in...the nucleolus”, and further refers to the finding that FITC-CTP-labeled nascent transcripts are present in the perinucleolar compartment, “a nuclear structure”. Dirks then goes on to describe microinjection into living cells of fluorescently labeled RNA molecules obtained by *in vitro* transcription, and notes that premRNA is localized in speckle domains (see page 4, col. 1-2). One of average skill in the art is aware that speckle domains are compartments in the nucleus enriched in splicing factors. Dirks further discusses efforts to efficiently deliver probes to desired target sites in cells, particularly in the nucleus: ”small sized probes were shown to accumulate in the nucleus”, ”ODNs...enter the nucleus by free diffusion, plasmid probes enter the nucleus by an energy-dependent mechanism; ”microinjected DNA fragments...were nearly immobile in the cell nucleus...the result of their binding to nuclear proteins” (see page 5, col. 2). Dirks further discusses use of phosphorothioate oligodeoxynucleotides and 2'-*O*-methyl-RNAs to study nuclear distribution of snRNAs, and discloses that distinct snRNA-specific probes accumulated variously in Cajal bodies, speckles and nucleoli (see page 6, col. 1 and Fig. 2). One of average skill in the art is aware that Cajal bodies, speckles and nucleoli are compartments within the eukaryotic nucleus. In discussing use of molecular beacons, Dirks discloses that such probes “open up as soon as they enter the nucleus”. Further discussion is directed to nuclear poly (A) RNA labeled with caged fluorochromes, and exposure of the nucleus to laser light, upon which the poly (A) RNA was shown to “move throughout the nucleus”.

The aforementioned disclosures in Dirks provide no teaching of labeling ribosomes, and in fact teach against labeling of ribosomes. That is, the labeled RNA species in Dirks could not possibly label ribosomes by association, since ribosomes are located in the cytoplasm and thus would not be labeled by RNA species localized in the nucleus or compartments thereof.

Furthermore, and as pointed out above, the disclosure in Dirks of Tsuji's FRET approach for detecting mRNA in the cytoplasm provides no teaching or suggestion of protein synthesis or labeling of ribosomes. Tsuji teaches against labeling of ribosomes or any other cytoplasmic component by the oligoDNA probes, since the article discloses that the results suggest that "most of the streptavidin-bound oligoDNAs did not have any specific interaction with other components in cells and were free within the cytoplasm" (see Tsuji page 3273, col. 1).

Based on the above, neither Rothschild nor Dirks render obvious claim 86 as amended, or the additional rejected claims. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C.103(a) be withdrawn.

Claims 86-107 are rejected under 35 U.S.C.103(a) as unpatentable over Kukhanova et al Molecular Biology Reports Vol. 1, No. 7/ September, 1974 pages 397-400 (hereinafter referred to as "Kukhanova"), in view of Odom et al Biochemistry 1990 Dec 4;29(48):10734 (hereinafter referred to as "Odom") or Paulsen et al Biochemistry, Vol. 25, No. 10, pages 2749, 1986, (hereinafter referred to as "Paulsen").

Kukhanova teaches binding of fluorescently labeled peptidyl-tRNA to ribosomes to study ribosome function and structure. However, Kukhanova provides no suggestion of a method comprising a step of analyzing emitted radiation so as to identify at least one protein being synthesized, as taught and claimed by the subject method. Further, Kukhanova contains no teaching of attaching a covalently bound label to ribosomes, nor that the experiments disclosed therein lead to covalent association between labeled tRNA and ribosome. In fact, Kukhanova teaches that hydrophobic interactions play an

important role in binding peptidyl-tRNA to the peptidyltransferase center (PTC) of ribosomes: "the ability of peptidyl-tRNA to become attached to the PTC of ribosomes increases as does hydrophobicity and is rather high even if the template is absent" (page 400, final sentence).

The deficiencies of Kukhanova are not remedied by either Odom or Paulsen. While Odom does teach use of covalently labeled ribosomal proteins and tRNAs, Odom does not suggest analysis of emitted signals so as to identify a protein being synthesized. Odom's primary conclusion is that the disclosed observations regarding tRNA movement and the binding capacity of the ribosome provide evidence in favor of the displacement model of peptide elongation on ribosomes and against the classical two-site model (see Discussion).

Furthermore, Odom's system cannot be used to detect a single ribosome as is provided by the present invention, since Odom requires corrections of fluorescent measurements in order to correct for incomplete binding of tRNA to ribosomes, and incomplete labeling of ribosomes (see page 10738).

Paulsen is directed to assessing the mechanism of translocation using fluorescently labeled peptidyl-tRNA to bind ribosomes. Like Kukhanova, Paulsen contains no teaching of attaching a covalently bound label to ribosomes, nor that the experiments disclosed therein lead to covalent association between labeled tRNA and ribosome. Rather, Paulsen relates to base pairing interactions within the tRNA-ribosome complex: "codon-anticodon interaction in the P site has been shown from several lines of evidence"; "the tRNA molecule, while it is bound to the E site, may adopt several conformation states on its way from the P site to the E site bound or unbound state, and that the codon-anticodon interaction does not take place in all of these states" (see page 2754, col. 2). Paulsen also does not suggest using analysis of emitted signals so as to identify a protein being synthesized. Rather, Paulsen uses the signals emitted from the system in order to assess the phases of the translocation reaction, characterized as occurring in three steps with different apparent rate constants (see Abstract).

Based on the above, Kukhanova, either alone or in combination with Odom or Paulsen does not render obvious claim 86 as amended, or the additional rejected claims. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C.103(a) be withdrawn.

In view of the above amendments and remarks, the application is now in condition for allowance and such action is respectfully requested. The examiner is invited to contact Applicants' undersigned representative if there is anything that will place the application in better condition for allowance.

Respectfully submitted,


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Dated: November 19, 2008

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